NATURAL HISTORY MISCELLANEA

Published by

The Chicago Academy of Sciences

Lincoln Park-2001 N. Clark St., Chicago 14, Illinois, U.S.A.

No. 178 December 15, 1961

Preliminary Experiments on the Role of the Cloacal Bursae in Hibernating Turtles

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The hypothesis has been proposed recently that the primary survival value that channeled evolution of the cloaca' bursae in turtles was respiration during aquatic hibernation and estivation (Smith and James, 1958) . Verification of existence of a respiratory function by the bursae in hibernating turtles would constitute strong support for such a hypothesis. Accordingly, tests designed to reveal presence or absence of such a function were made between June, 1958, and March, 1959, more or less duplicating temperatures and inanition occurring during natural hibernation, and controlling certain modes of respiration.

All turtles used in these experiments were donated by the Chicago Zoological Park and the Lincoln Park Zoo. Upon receipt they were placed for either one or eight weeks in a large tank at a temperature ranging between 13° C. and 16° C. (55° F.-60° F.) . This period permitted a certain degree of acclimation to a moderately low temperature, and elimination of food and wastes from the digestive tract. No food was offered at any time.

The specimens were then placed in large stone crocks in a constant-temperature cold room at 1° C.-2° C., where they were exposed to one of eight combinations of three controlled variables. Part were kept submerged under water (WU) at all times, and the remainder was kept in shallow water one-fourth to one-half inch in depth **(WO)** . A one-fourth inch mesh hardware cloth partition was suspended near the middle of each crock, and water kept at a level covering the partition to a depth of one-fourth to one-half inch. The submerged turtles were kept below the wire partition by the weight of the **WO** turtles or of stones kept above the partition.

Some of each of these groups had the cloaca obstructed (CP) by insertion of the largest possible cork into the cloaca, subsequently either sewing the anus shut (Exp. 1) or anchoring the cork at the vent by

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stitches looped through the cork and margins of the anus (Exp. 2). Others were untreated insofar as the cloaca was concerned (CO).

Finally some of both the WO and WU groups had the head capped (HC) by means of a heavy, slender, straightsided (not bulbous) rubber balloon fitting sufficiently tight to prevent air passage, but not tight enough to interfere with circulation. Others were kept intact, without head cap (HO).

Thus all specimens were subjected to one of the following eight combinations of conditions : WO-CO-HO, WO-CP-HO, WO-CO-HC, WO-CP-HC, WU-CO-HO, WU-CP-HO, WU-CO-HC, WU-CP-HC.

Comparative resistance of turtles to these conditions was determined by the number of days of survival. The turtles were examined at irregular intervals of two to twenty days ; the survival period for each specimen was recorded as the number of days between initiation of exposure to extreme cold and the last date upon which the animal was observed alive.

A few turtles upon a given inspection showed clear sign of death, being either completely limp or stiff as though with rigor mortis, but most were enigmatic as they became quiescent and gave indication of life only upon repeated stimulation.

Inversion and tapping on plastron was sufficient to produce movement in most cases where life remained but was at low ebb. Specimens from which these stimuli elicited no response were removed and the limbs flicked strongly with the finger ; slow withdrawal movements indicated persistence of life. Failure of this procedure to produce a response was followed by placing the turtle on its back on the floor and administering light blows on the plastron and carapace. The turtle was assumed to be dead if no response appeared after five or ten minutes.

After a few hours at 1-2° C. most turtles moved very little, even when disturbed by inspection, but considerable variation was noted. Some turtles remained responsive for weeks and moved about rather actively upon any sort of disturbance ; the painted turtles *(Chrysemys)* were particularly resistant to the numbing effect of cold, as shown by the data given herein and by their exceptional activity.

Death of the turtles is assumed to have been caused by the direct effect of the cold, or by its effect in combination primarily with inefficiency of respiration. Inanition probably was not an important factor. Turtles in the first experiment were held without feeding at 13-16° C. for a longer period (eight weeks) than were those in the second experiment (one week) before exposure to extreme cold and, therefore, the effect of starvation, if of critical importance, would be evident in the first group. On the contrary, mean survival rates in the first experi-

exceeded them. Starvation possibly was a critical factor in the cases of maximum lengths of survival, but the very low rate of metabolism occurring at low temperatures, and the known survival for several months under both natural and artificial conditions suggest that starvation rarely would be involved importantly in death under the conditions of these experiments.

The large size of the cold room (48 x 46 ft., ceiling 9 ft. 8 in.) and its moderately frequent use (opening and closing of doors) assured maintenance of acceptable gaseous differentials between environment and organism more or less approximately those of natural conditions. Therefore, death from anoxia could result only within experimentally controlled limits.

The most important possible uncontrolled factor of experimental source in causing death of the turtles was accumulation of possibly noxious solutes within the cloaca of the animals, or in the water in which they were partially or fully immersed. The water was not changed during the course of the entire experiment, although fresh water was added at intervals to offset evaporation and volume loss when animals were removed. Since the crocks were of five and ten gallon capacity, the water content was about three and six gallons, respectively. One to four turtles were placed in small crocks, one to eight in larger crocks. No discoloration or odor was noted in the water when discarded at the end of the experiments, but no test of toxicity was made. It is doubtful that the water ever became critically toxic, and since in WU turtles there was no significant difference in survival between the CP and CO groups it is doubtful that uremia was an importantly critical factor in cause of death.

RESULTS

Days of survival of each of the 58 specimens entered in the experiment are given in the accompanying table. Those animals tested in the first experiment (cold treatment started June 3, 1958) and second experiment (cold treatment started August 27, 1958) are indicated by the parenthetic figures (1) and (2) , respectively, following the entry for survival days. Means for sets represented by two or more tests are given as a parenthetic figure of two or three digits, below a horizontal line under the last entry for experimental data. The species represented were *Chrysemys picta,* 31 (I) ; *Pseudemys scripta,* 20 (II) ; *Pseudemys floridana,* 2 (III) ; *Emydoidea blandingi,* 1 (IV) ; *Terrapene Carolina,* 2 (V) ; *Graptemys pseudogeographica,* 1 (VI) ; and *Deirochelys reticularia,* 1 (VII) . No particular effort was made to secure equal numbers of each species, under the assumption that the data for CO specimens of species with well-developed cloacal bursae would be interchangeable within the group, and that data for

interchangeable. These assumptions proved erroneous, as the variation between species within either the CP or CO groups (with or without bursae) exceeded that within a given species between the CP and CO groups. Therefore, the data for species III-VII are of limited value.

With respect to these species, however, three observations are noteworthy. First, the single specimen of *Emydoidea* survived the least time of all individuals under water, suggesting that either *Emydoidea* may normally hibernate on land, or that the bursae are of critical respiratory importance in this species. Both Carr (1952) and Cahn (1937) , however, state that at least some specimens of the semibox turtle hibernate under water ; some examples hibernate on land, but the species is clearly not dependent upon hibernation on land, and it is doubtful that there is a difference between individuals of the species in adjustment to underwater or terrestrial hibernation. Presumably, therefore, the bursae may be of critical importance in underwater hibernation in this species.

It is also of interest in connection with *Terrapene,* the only group tested lacking bursae *(carolina* has only blind vestiges, clearly non-functional) , that the WU specimen (CO-HO) survived 62 days longer than most other WU specimens, excluding *Chrysemys.* The single WO specimen survived 90 days, at which time it was removed and released after recovery. Bursae clearly are not likely of importance in underwater hibernation in this species.

Thirdly, the data suggest that the WU-CP-HO survival rates for *Pseudemys floridana, Graptemys* and *Deirochelys* will prove, with larger series, to be of the same order of magnitude as that for *Pseudemys scripta.* That order may be somewhere between the extreme exemplified by *Emydoidea,* which may be highly susceptible to WU-CP-HO, and the extreme exemplified by *Chrysemys,* which is highly resistant to WU-CP-HO.

The most important data are those for the species represented by the largest series of specimens. Unfortunately, the eight sets so disperse the otherwise respectable series of 51 specimens (31 for I, 20 for II) that most sets contain only four specimens, some two and only one as many as five.

The only statistical tests made were analyses of variance within each of four sets of data the two WO groups and the two WU groups in the species I and II. The probability of non-heterogeneity in the four sets of data in WO I is very high ; the F value is 1.53, whereas an F value of at least 3.71 would be required for a borderline significance of .95, or 6.55 for a definite significance of .99. These three comparatives for the four sets in WO II are 5.47, 5.41, 12.1 ; for WU I 0.321, 3.49, 5.95 ; and for WU II 3.86, 4.35, 8.45. Variance

confidence level and only one (WO II) is acceptable as heterogeneous at the .05 level. The statistical insufficiency of these data is believed to be due primarily to small size of the samples in the tests ; examination

TABLE 1. Turtle Survival at 1° C - 2° C
Under Eight Combinations of Variables*

	I	II	III	IV	V	VI	VII
WO-CO-HO	159 (2) 197 (2) (178)	100 (2) 105 (1) 159 (2) (121)	112(1)				
WO-CP-HO	76 (2) 110 (2) 150 (2) 156 (1) (123)	76 (2) 143 (1) (110)			90+(1)		
WO-CO-HC	72 (2) 129 (2) 140 (2) 160 (2) (125)	34 (2) 43 (2) (37)					
WO-CP-HC	97 (2) 108 (2) 119 (2) 160 (2) (121)	34 (2) 34 (2) (34)					
WU-CO-HO	37 (1) 91 (2) 91 (2) 119 (1) (85)	27 (2) 34 (2) 42 (1) 63 (1) (42)			62(1)		
WU-CP-HO	27 (2) 64 (2) 70 (2) 70 (2) 176 (1) (81)	43 (2) 63 (1) 63 (1) (56)	34(2)	10(1)		43(2)	27(2)
WU-CO-HC	72 (2) 72 (2) 101 (2) 112 (2) (89)	20 (2) 27 (2) (24)					
WU-CP-HC	63 (2) 63 (2) 103 (2) 103 (2) (83)	27 (2) 27 (2) (27)					

*See text for explanation of symbols.

of the raw data is strongly suggestive of a number of relationships but, unfortunately, the sample sizes of the experiments now complete will permit no more than erection of more hypotheses instead of providing an answer to the hypothesis the experiments were undertaken to test. Discussion of the relationships and correlations suggested by the completed experiments follows.

As might be expected, under-water hibernation cannot be tolerated as long as hibernation out of water, other factors being equal. The comparative means (WU and WO respectively) are as follows : CO-HO I 85-178, II 42-121, IV 62-90+ ; CP-HO I 82-123, II 56-110; CO-HC I 89-125, II 24-37 CP-HC I 83-121, II 27-34. No exceptions to this hypothesis, on the basis of means, exist in the data obtained, although there is an overlap of ranges in two pairs of sets.

One other invariable relationship is the greater average survival of *Chrysemys* under any given set of conditions. The comparative means of *Chrysemys* and *Pseudemys scripta,* respectively, are as follows : WO-CO-HO 178-121; WO-CP-HO 123-110 ; WO-CO-HC 125-37 ; WO-CP-HC 121-34 ; WU-CO-HO 85-42 ; WU-CP-HO 81-56 ; WU-CO-HC 89-24; and WU-CP-HC 83-27. Overlap of 1 digit occurs in ranges in two sets of data, of 27 (in total range of 73) in one set ; no overlap occurs in others. Furthermore the means for *Chrysemys* exceed those for all other species under the same sets of conditions. It may be hypothesized safely that *Chrysemys,* therefore, is more resistant to cold than any other species with which it has been compared. The geographic range of *Chrysemys ys* strongly supports this hypothesis, since the species occurs farther north than any other tested.

Not all of the marked contrasts evident between *Chrysemys* and *Pseudemys scripta* can be explained simply on the basis of difference in resistance to cold. CP-HO *Pseudemys,* both WO and WU, are not much different from CP-HO *Chrysemys* in survival potential, but HC specimens, both WO and WU, have a strongly reduced potential, with little difference whether CP or CO. Evidently, *Pseudemys* may rely heavily upon buccal respiration, both WU and WO. These contrasts, it may be noted, are the two that came the closest to statistical acceptability.

On the other hand, in *Chrysemys,* although the mean for WO-CO-HO is higher (178) than for any CP or HC combination, the WO-HC groups (121, 125) are not even suggestively different from the WO-CP-HO group (123) . Similarly, the WU *Chrysemys* suggest no particular HC sensitivity. Evidently buccal respiration in *Chrysemys,* although perhaps of some importance, is not of the critical value that it is in *Pseudemys,* as is borne out by the greater statistical probability of heterogeneity in *Pseudemys* within the sets WO and WU, as opposed to high probability of homogeneity in *Chrysemys*

within the same sets. In fact, the ability of *Chrysemys* to survive so well without either buccal or cloacal respiration poses a problem that seemingly can be answered solely by assuming that integumentary respiration supplies the need to an important degree. Whatever the alternative device may be, it seems not to be operative in *Pseudemys.*

SUMMARY

1. Respiratory needs and relative importance and identity of vehicles for mediation of external respiration vary, at present unpredictably, among turtles even of closely related genera.

2. Under the experimental conditions established, statistical means for survival invariably were greater in out-of-water hibernation than in under-water hibernation, other factors being equal.

3. Means for survival in *Chrysemys,* under the experimental conditions established, invariably were greater than in other species ; a probable correlation is the greater range northward of this species as compared with others tested.

4. In *Chrysemys* hibernating in very shallow water both cloacal and buccal respiration seem to be of some importance, but neither is of critical importance.

5. In *Chrysemys* hibernating completely under water there is no evidence of an important contribution to respiration of either cloacal or buccal organs ; some other device provides at least a part of respiratory needs.

6. Disregarding difference in resistance to cold, *Chrysemys* has a greater survival potential, with cloacal and buccal respiration blocked, than *Pseudemys,* which appears to lack, or have poorly developed, a supplementary respiratory device.

7. The cloacal bursae of *Pseudemys scripta,* under the experimental conditions established, seem to make no important contribution to respiration in hibernation either under or out of water.

8. *Pseudemys scripta,* under the experimental conditions established, appears to rely heavily upon buccal respiration in hibernation both under and out of water.

9. *Terrapene,* despite natural absence of functional bursae, seemingly is moderately tolerant to underwater hibernation more resistant than species (excluding *Chrysemys)* with bursae but with the cloaca plugged.

10. Of species tested with bursae but the cloaca plugged and buccal respiration not altered, *Emydoidea* is least tolerant, *Chrysemys* most tolerant, and the remainder *(Pseudemys, Graptemys, Deirochelys)* of intermediate tolerance to underwater hibernation.

11. The significance of the apparent susceptibility of *Emydoidea* to underwater hiberation with the cloaca plugged (and buccal respira-

under water in nature, it may rely extensively upon the bursae for respiration.

12. Critical importance of cloacal bursae in respiration during underwater hibernation still lacks experimental proof ; it remains a possible factor in the physiology of at least some living species and, irrespective of present function, it may well have been a selective factor involved in establishment of bursae in evolution of certain population lines of turtles.

13. Buccal respiration in hibernating turtles is not always of critical importance either under or out of water, even when cloacal respiration is prevented ; importance of buccal respiration varies with species.

14. The organ important for respiration in the species having high survival potential even in absence of both buccal and cloacal respiration is presumed to be the skin.

ACKNOWLEDGMENTS

For indispensable assistance we are indebted to Mr. R. Marlin Perkins of Lincoln Park Zoo, and to Mr. Robert Bean, Mr. Robert Snedigar, and Dr. George Rabb, of Chicago Zoological Park, for viding experimental facilities in the University of Illinois Vivarium ; providing all experimental animals ; to Dr. S. C. Kendeigh for pro- to the Horticulture Department, University of Illinois, for permission to use a cold room in the Horticulture Field Laboratory; to Mr. Clarence Rund for help in handling the turtles in the cold room ; and to Dr. Robert Bader and Dr. James Huheey for critical comments and advice with statistical problems.

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